

66. (New) The method of claim 65, wherein the other enzymes are selected from the group consisting of an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, haloperoxidase, invertase, laccase, lipase, mannosidase, oxidase, pectinolytic enzyme, peptidoglutaminase, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

#### REMARKS

Claims 1-10, 12, 13, 15-18, 22, 25, 28, and 32 have been cancelled without prejudice or disclaimer. New claims 34-66 have been added and are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

#### **I. The Rejection of Claims 1-10, 12-13, 15-18, 22, 25, 28 and 32 under 35 U.S.C. § 112, Second Paragraph**

Claims 1-10, 12-13, 15-18, 22, 25, 28 and 32 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several grounds.

Ground 1: The Office Action states that claim 7 lacks proper antecedent basis in claim 6 for "the aerobic bacteria". Claim 7 has been cancelled, but new claim 40, corresponding to cancelled claim 7, recites "bacterium" to provide a proper antecedent basis.

Ground 2: The Office Action states that claim 8 lacks proper antecedent basis for "the anaerobic bacteria". Claim 8 has been cancelled but new claim 41, corresponding to cancelled claim 8, recites "bacterium" to provide a proper antecedent basis.

Ground 3: The Office Action states that Claim 18 lacks proper antecedent basis in claim 1 for "the effective concentration of one or more acylases" because Claim 1 pertains to "an effective amount of a composition comprising..." Claim 1 has been cancelled but new claim 34, which corresponds to claim 1, recites "an effective amount of one or more acylases".

Ground 4: The Office Action states that Claim 28 lacks proper antecedent basis in claim 1 for "the surface is contacted with the one or more acylases" because Claim 1 pertains to "contacting the surface with an effective amount of a composition comprising..." Claim 1 has been cancelled but new

claim 34, which corresponds to claim 1, recites "an effective amount of one or more acylases".

Ground 5: The Office Action states that claim 7 is confusing in that the distinction between *Pseudomonas* and *Burkholderie* is unclear. This rejection is respectfully traversed. *Burkholderie* is a relatively new genus where several *Pseudomonas* strains have been reclassified as *Burkholderie*. See Yabuuchi *et al.*, 1993, *Int. J. Syst. Bacteriol.* 43: 398-399 and Kersters *et al.*, 1996, *Systematic and Applied Microbiology* 19: 465-477.

Ground 6: The Office Action states that Claim 16 is confusing in that the distinction between *Yarrowia* and *Candida* is unclear. This rejection is respectfully traversed. *Yarrowia* and *Candida* are both dimorphic fungi meaning they can grow as budding yeast or as mycelia. However, *Candida* has no sexual cycle while *Yarrowia* does. *Candida* is diploid while *Yarrowia* is haploid. See Herrero *et al.*, 1999, *Microbiology* 145: 2727-2737, a copy of which is attached.

Ground 7: The Office Action states that Claim 18 is vague and indefinite in that the nature of one or more acylases effective to eliminate biofilms at a concentration of 0.001 g of acylase per kilogram of water is not ascertainable, even when interpreting the claim in light of the specification and that the activity of the acylase intended is not indicated. This rejection is respectfully traversed. One of ordinary skill in the art would recognize that the effective amount of the one or more acylases can depend on the acylase(s), *e.g.*, specific activity, and the period of time desirable for degrading a microbially-produced lactone(s). High amounts of the enzyme(s) will likely require shorter times of treatment while low amounts longer times. Moreover, the amount of biofilm to be degraded or prevented could vary based on the surface and environment in which the biofilm is found. One skilled in the art would understand that the amount of acylase to use in a commercial setting is better expressed as gram(s) of acylase per kilogram of water.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

## **II. The Rejection of Claims 1-10, 12-13, 18, 22, 25, 28, and 32 under 35 U.S.C. § 102**

Claims 1-10, 12-13, 18, 22, 25, 28, and 32 are rejected under 35 U.S.C. § 102(b) as being anticipated by Wiatr (U.S. Patent No. 4,936,994). The Office Action states:

Wiatr discloses a method of eliminating microbial biofilms on surfaces wherein the surfaces are contacted with an enzyme composition comprising acylases such that the degradation of a lactone results in elimination of biofilm. See, *e.g.*, Table II, wherein the effective use of the acylases neutral protease and alkaline proteases is disclosed. The use of other enzymes is noted. At least temperature and pH parameters are within the required ranges. Similarly, it is presumed that at least the lower level of acylase

concentration is met by the reference, particularly in the absence of evidence to the contrary.

This rejection is respectfully traversed.

Under the standard required for anticipation under 35 U.S.C. § 102, the cited prior art reference is required to disclose every element of the claimed invention. *Lewmar Marine Inc. v. Barient Inc.*, 3 USPQ2d 1766 (Fed. Cir. 1987).

Wiatr discloses methods for removing slime from slime-covered surfaces of cooling towers which comprises contacting the surfaces with an effective amount of a composite enzyme preparation consisting of cellulase, beta-glucanase, alpha-amylase, neutral protease, and alkaline protease. However, Wiatr does not disclose methods for preventing or removing biofilm on a surface, comprising contacting the surface with an effective amount of one or more acylases and a carrier to degrade a lactone produced by one or more microorganisms, wherein the degradation of the lactone prevents or removes the biofilm, as claimed herein.

In the methods of the present invention, one of ordinary skill would recognize that an "acylase" is a hydrolytic activity which catalyzes the deacylation of N-acylated amino acids or amines. Acylases (amidases, aminoacylases, etc.) belong to EC 3.5 subclass, acting on nonpeptide C-N bonds. Proteases and peptidases (neutral/alkaline-proteases, etc) belong to EC 3.4 subclass, acting on peptide C-N bonds. See International Union of Biochemistry and Molecular Biology (IUBMB) Enzyme Nomenclature; <http://www.chem.qmul.ac.uk/iubmb/>. Neutral and alkaline proteases are, therefore, not acylases.

For the foregoing reason, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 102. Applicants respectfully request reconsideration and withdrawal of the rejection.

### **III. The Rejection of Claims 1-10, 12-13, 15, 17-18, 22, 25, 28, and 32 under 35 U.S.C. § 102**

Claims 1-10, 12-13, 15, 17-18, 22, 25, 28, and 32 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Hollis *et al.* (U.S. Patent No. 5,411,666). The Office Action states:

Hollis *et al.* discloses a method of eliminating microbial biofilms on surfaces wherein the surfaces are contacted with an enzyme composition comprising acylases such that the degradation of a lactone results in elimination of biofilm. See, *e.g.*, Examples 1 and 7 wherein the effective use of the acylases, *i.e.*, acidic and alkaline proteases are used to eliminate biofilm. The use of surfactants and other enzymes is noted. At least temperature and pH parameters are within the required ranges. Similarly, it is presumed that at least the lower level of acylase concentration is met by the reference, particularly in the absence of evidence to the contrary.

This rejection is respectfully traversed.

Hollis *et al.* discloses methods of removing a biofilm from a solid substrate or preventing buildup of a biofilm on a solid substrate, said biofilm being formed by at least one sessile microorganism in a water system comprising the step of contacting a water system in recognized need of such removal or prevention with a composition consisting essentially of (1) at least one acidic protease or alkaline protease, (2) at least one glucoamylase or alpha amylase, and (3) at least one surfactant, said combination of (1), (2), and (3) being capable of destroying polysaccharide material which surrounds the sessile microorganisms. However, Hollis *et al.* do not disclose methods for preventing or removing biofilm on a surface, comprising contacting the surface with an effective amount of one or more acylases and a carrier to degrade a lactone produced by one or more microorganisms, wherein the degradation of the lactone prevents or removes the biofilm, as claimed herein.

In the methods of the present invention, one of ordinary skill would recognize that an "acylase" is a hydrolytic activity which catalyzes the deacylation of N-acylated amino acids or amines. Acylases (amidases, aminoacylases, etc.) belong to EC 3.5 subclass, acting on nonpeptide C-N bonds. Proteases and peptidases (acid-/neutral-/alkaline-proteases, etc) belong to EC 3.4 subclass, acting on peptide C-N bonds. See IUBMB Enzyme Nomenclature; <http://www.chem.qmul.ac.uk/iubmb/>. Acidic and alkaline proteases are, therefore, not acylases.

For the foregoing reason, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 102. Applicants respectfully request reconsideration and withdrawal of the rejection.

#### **IV. The Rejection of Claims 1-10, 12-13, 15-18, 22, 25, 28, and 32 under 35 U.S.C. § 102**

Claims 1-10, 12-13, 15-18, 22, 25, 28, and 32 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Berka *et al.* (WO 00/28043). The Office Action states:

Berka *et al.* discloses a method of eliminating microbial biofilms on surfaces wherein the surfaces are contacted with an enzyme composition comprising acylases such that the degradation of a lactone results in elimination of biofilm. See, e.g., page 36, lines 25-30 wherein the effective use of the acylases is disclosed. At least temperature and pH parameters are within the required ranges. Similarly, it is presumed that at least the lower level of acylase concentration is met by the reference, particularly in the absence of evidence to the contrary.

This rejection is respectfully traversed.

Berka *et al.* disclose a method for preventing biofilm development on a liquid-solid interface by one or more microorganisms, comprising administering an effective amount of one or more polypeptides having

lactonohydrolase activity and a carrier to the liquid-solid interface to degrade one or more lactones produced by the one or more microorganisms, wherein the one or more lactones are involved in the formation of the biofilm. However, Berka *et al.* do not disclose methods for preventing or removing biofilm on a surface, comprising contacting the surface with an effective amount of one or more acylases and a carrier to degrade a lactone produced by one or more microorganisms, wherein the degradation of the lactone prevents or removes the biofilm, as claimed herein.

In the methods of the present invention, one of ordinary skill would recognize that an "acylase" is a hydrolytic activity which catalyzes the deacylation of N-acylated amino acids or amines. Acylases (amidases, aminoacylases, etc.) belong to EC 3.5 subclass, acting on nonpeptide C-N bonds. Lactonohydrolases (lactonases, lacton hydrolases, etc) belong to EC 3.1.1 sub-subclass of EC 3.1 subclass, acting on carboxylic ester C-O bonds. See IUBMB Enzyme Nomenclature; <http://www.chem.qmul.ac.uk/iubmb/>. Lactonohydrolases are, therefore, not acylases.

For the foregoing reason, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 102. Applicants respectfully request reconsideration and withdrawal of the rejection.

#### **V. The Rejection of Claims 1-10, 12-13, 15-18, 22, 25, 28, and 32 under 35 U.S.C. § 103**

Claims 1-10, 12-13, 15-18, 22, 25, 28, and 32 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Wiatr (U.S. Patent No. 4,936,994) taken with Hollis *et al.* (U.S. Patent No. 5,411,666) and Allison *et al.* (*FEMS Microbiology Letters*, 1998, 167: 179-184). The Office Action states:

[O]ne of ordinary skill in the art would have had a reasonable expectation of success in using an acylase as taught by Hollis *et al.* and Wiatr to eliminate a variety of biofilms in industrial and therapeutic settings. It would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the process Wiatr and Hollis *et al.* by using enzymes capable of hydrolyzing N-acyl-hexanoyl homoserine, such as acylases and in particular, lactonohydrolases, for the expected benefit of decreasing biofilms on surfaces in both industrial and therapeutic applications, such as biofilms of piping, heat exchangers, food process, potable water systems and in the treatment of dental caries, periodontal disease, cystic fibrosis, etc.

This rejection is respectfully traversed.

The Examiner has the initial burden of establishing a *prima facie* case of obviousness. A finding of obviousness under § 103 requires a determination of the scope and content of the prior art, the differences between the claimed invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. Deere*, 383 US 1

(1966). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion that the combination be made. *In re Stencel*, 828 F2d 751, 4 USPQ2d 1071 (Fed. Cir. 1987).

The present invention relates to methods for preventing or removing biofilm on a surface, comprising contacting the surface with an effective amount of one or more acylases and a carrier to degrade a lactone produced by one or more microorganisms, wherein the degradation of the lactone prevents or removes the biofilm.

Applicants submit that Wiatr, Hollis *et al.*, and Allison *et al.*, alone or in combination, do not teach or suggest the methods of the present invention. There must be something in the prior art as a whole which suggests the desirability, and thus the obviousness, of making the combination.

Wiatr discloses methods for removing slime from slime-covered surfaces of cooling towers which comprises contacting the surfaces with an effective amount of a composite enzyme preparation consisting of cellulase, beta-glucanase, alpha-amylase, neutral protease, and alkaline protease.

Hollis *et al.* discloses methods of removing a biofilm from a solid substrate or preventing buildup of a biofilm on a solid substrate, said biofilm being formed by at least one sessile microorganism in a water system comprising the step of contacting a water system in recognized need of such removal or prevention with a composition consisting essentially of (1) at least one acidic protease or alkaline protease, (2) at least one glucoamylase or alpha amylase, and (3) at least one surfactant, said combination of (1), (2), and (3) being capable of destroying polysaccharide material which surrounds the sessile microorganisms.

Allison *et al.* discloses that (1) cell-cell signals such as homoserine lactones are associated with the formation of *P. fluorescens* biofilms, (2) the exopolymer lyase degradation of exopolymers has a specific role in the detachment of cells under starvation conditions, and (3) while short chain (C<sub>6</sub>) exogenous homoserines can trigger such responses in *P. fluorescens*, its own signal substance is likely to possess a longer (>C<sub>8</sub>) fatty acyl chain.

Applicants assert that there is no motivation to combine the cited references because the references do not suggest or teach methods for preventing or removing biofilm on a surface with acylase(s), as claimed herein. While each of the cited references suggest the involvement of protease, lactonohydrolase, and exopolymer lyase in the degradation of biofilm, none of the references suggest or teach the use of an acylase. The Office Action mistakenly suggests that the cited enzymes are acylases. Acylases (amidases, aminoacylases, etc.) belong to EC 3.5 subclass, acting on nonpeptide C-N bonds. Proteases and peptidases (neutral/alkaline-proteases, etc) belong to EC 3.4 subclass,

acting on peptide C-N bonds. Lactonohydrolases (lactonases, lacton hydrolases, etc) belong to EC 3.1.1 sub-subclass of EC 3.1 subclass, acting on carboxylic ester C-O bonds.

Applicants submit, therefore, that the references cited by the Examiner do not contain the requisite teaching, and therefore cannot be combined to support the obviousness rejections of the present claims.

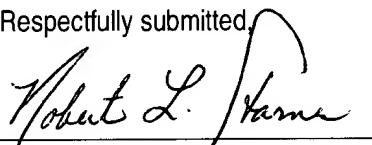
For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 103(a). Applicants respectfully request reconsideration and withdrawal of the rejection.

## **VI. Conclusion**

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Date: August 28, 2003

Respectfully submitted,

A handwritten signature in cursive script, reading "Robert L. Starnes", written over a horizontal line.

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